U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-295 376

Toxicity Evaluation of a Complex Metal Mixture to the Shoftshell Clam 'Mya arenaria'

(U.S.) Environmental Research Lab., Narragansett, RI

20 May 77

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. PB 295376
EPA-600/J-77-149	JOURNAL ARTICLE	

4. TITLE AND SUBTITLE
Toxicity Evaluation of a Complex Metal Mixture to the Softshell Clam Mya arenaria

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Ronald Eisler

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory
Narragansett, RI 02882

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory
Narragansett, RI 02882

13. TYPE OF REPORT AND PERIOD COVERED
Journal reprint

14. SPONSORING AGENCY CODE
EPA/600/05

15. SUPPLEMENTARY NOTES
REFERENCE: MARINE BIOLOGY, 43:265-276, 1977

16. ABSTRACT

Adults of the softshell clam *Mya arenaria* were continuously subjected to a flowing raw seawater solution containing a mixture of salts of manganese, zinc, lead, nickel, copper, and cadmium. Final calculated concentrations, in $\mu g\ l^{-1}$, of the toxicant solution were 7200 Mn, 2500 Zn, 70 Pb, 50 Ni, 50 Cu and 1 Cd; these concentrations approximated highest measured levels within surficial interstitial sediment waters from mid-Narragansett Bay, Rhode Island. *M. arenaria* were also subjected to a 20% solution, i.e., 1440 Mn, 500 Zn, 14 Pb, 10 Ni, 10 Cu, and 0.2 $\mu g\ l^{-1}$ Cd. One study was conducted for 112 days in winter at 0° to 10°C and another for 16 days in summer at 16° to 22°C. In the winter study, all clams exposed to a 100% solution died between the 4th and 10th week; soft parts of survivors at 6 weeks contained about 19 times more Pb, 15 x more Zn, 12 x more Cu, 10 x more Mn, 3 x more Ni and 0.1 x more Cd than controls; relatively minor changes in whole body elemental content of Ca, Cr, Fe, K, Mg, Na, Sr, and V were observed. Clams exposed to a 20% solution during winter survived the 112 day study; at that time these contained about 5 x more Cu, 4 x more Mn, 3 x more Zn and about 2 x more Pb than controls; comparatively minor changes were observed in other elements examined. In the summer study, all *M. arenaria* subjected to the 100% solution died between 6 and 14 days; survivors from this group at 7 days contained about 25 x more Pb, 13 x more Cu, 11 x more Zn, 7 x more Mn, and 3 x more Ni than controls; other changes in elemental content were not as pronounced. Mortality in the 20% group during summer was slightly higher than controls during the 16 day study; at 14 days survivors from this group contained about 12 x more Mn, 7 x more Pb, 7 x more Zn, 4 x more Cu, and 3 x more Ni than controls. Survival and bioaccumulation patterns were not altered through feeding a supplemental diet of algae. The significance of these findings are discussed in terms of potential environmental perturbations, especially local dredging practices.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Marine Biology, Mollusca, Metals, Clams, Pollution, Physiology, Toxicity, Strontium, Vanadium, Manganese, Zinc, Lead, Nickel, Copper, Calcium, Chromium, Iron, Potassium, Magnesium, Sodium		

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release Unlimited		11
	20. SECURITY CLASS *(This page)*	22. PRICE
		A02-A01

Marine Biology 43, 265-276 (1977)

MARINE BIOLOGY
by Springer-Verlag 1977

EPA-600/J-77-149
JOURNAL ARTICLE

Toxicity Evaluation of a Complex Metal Mixture to the Softshell Clam *Mya arenaria*

R. Eisler

U.S. Environmental Protection Agency, Environmental Research Laboratory; Narragansett, Rhode Island, USA

Abstract

Adults of the softshell clam *Mya arenaria* were continuously subjected to a flowing raw seawater solution containing a mixture of salts of manganese, zinc, lead, nickel, copper, and cadmium. Final calculated concentrations, in $\mu g\ l^{-1}$, of the toxicant solution were 7200 Mn, 2500 Zn, 70 Pb, 50 Ni, 50 Cu and 1 Cd; these concentrations approximated highest measured levels within surficial interstitial sediment waters from mid-Narragansett Bay, Rhode Island. *M. arenaria* were also subjected to a 20% solution, i.e., 1440 Mn, 500 Zn, 14 Pb, 10 Ni, 10 Cu, and 0.2 $\mu g\ l^{-1}$ Cd. One study was conducted for 112 days in winter at 0° to 10°C and another for 16 days in summer at 16° to 22°C. In the winter study, all clams exposed to a 100% solution died between the 4th and 10th week; soft parts of survivors at 6 weeks contained about 19 times more Pb, 15 x more Zn, 12 x more Cu, 10 x more Mn, 3 x more Ni and 0.1 x more Cd than controls; relatively minor changes in whole body elemental content of Ca, Cr, Fe, K, Mg, Na, Sr, and V were observed. Clams exposed to a 20% solution during winter survived the 112 day study; at that time these contained about 5 x more Cu, 4 x more Mn, 3 x more Zn and about 2 x more Pb than controls; comparatively minor changes were observed in other elements examined. In the summer study, all *M. arenaria* subjected to the 100% solution died between 6 and 14 days; survivors from this group at 7 days contained about 25 x more Pb, 13 x more Cu, 11 x more Zn, 7 x more Mn, and 3 x more Ni than controls; other changes in elemental content were not as pronounced. Mortality in the 20% group during summer was slightly higher than controls during the 16 day study; at 14 days survivors from this group contained about 12 x more Mn, 7 x more Pb, 7 x more Zn, 4 x more Cu, and 3 x more Ni than controls. Survival and bioaccumulation patterns were not altered through feeding a supplemental diet of algae. The significance of these findings are discussed in terms of potential environmental perturbations, especially local dredging practices.

Introduction

Although there is a growing body of information on the toxicity of heavy metals salts to economically important species of marine bivalve molluscs (Eisler, 1973; Eisler and Wapner, 1975; Eisler, 1977), published information on biological effects of mixtures of metal salts on this group under controlled conditions is absent. Only limited data on this subject are avaiblable for other assemblages of aquatic taxa (Lloyd, 1961; Sprague, 1964a, b; Sprague and Ramsey, 1965; Cusick, 1967; Eaton, 1973; Eisler and Gardner, 1973; Gray and Ventilla, 1973), and it is noteworthy that all investigators concluded that behavior of the mixture was not necessarily predictable on the basis of action of individual components. It is also noted that each of the preceding studies employed a maximum of three metals, chosen from a list comprising Cd, Cu, Hg, Pb, and Zn, with final concentrations of individual metals in the mixture having little relation to concentrations or ratios encountered under field conditions.

Recent studies (Eisler *et al.*, in review; in press; and unpublished data) on heavy metal distributions in marine biota and non-biological matrices from the vicinity of an abandoned electroplating plant demonstrated elevated lev-

els of manganese, zinc, lead, copper, nickel, cadmium and other metals in all parameters examined. Accumulations were most pronounced among clams, *Pitar morrhuana*, taken at stations nearest plant outfalls; in addition, there was a measurable decrease in a number of mollusc species, including clams, at those locations. However, clams at all stations did not significantly reflect the metal composition of their surrounding substrate, i.e., sediments, sediment interstitial waters, water column, except for Mn and Zn in sediments. Since this study area, located in mid-Narragansett Bay, Rhode Island, was periodically and extensively dredged in order to enlarge existing ship channels, the possibility existed that metals released from sediment interstitial waters as a result of dredging and other perturbations might constitute one source of the elevated metal content observed in clams and the decrease in species abundance. The present account explores this possibility under laboratory conditions using adults of the softshell clam *Mya arenaria*, and a mixture of salts of Mn, Zn, Pb, Cu, Cd and Ni. Concentrations of individual metals in the mixture approximated the highest levels of each found in surficial sediment interstitial waters from mid-Narragansett Bay (Eisler *et al.*, unpublished data). Specifically, it reports on effects upon *M. arenaria* of a supplemental algae diet (add, no-add) and three dose levels (none, 20%, 100%) of the metal mixture tested on survival and whole-body residues of 14 elements (Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, V, Zn) during immersion in flowing raw seawater under ambient winter and summer regimes.

Materials and Methods

General

All studies were conducted aboard the Environmental Protection Agency Research Barge located in Jerusalem, Rhode Island. The barge is moored approximately 20 m offshore at a water depth of 7 m in the tidal channel separating Jerusalem from Galilee, Rhode Island.

Test Containers

Six rectangular fiberglass troughs each of 100 l capacity were used for acclimatization and bioassay purposes. Individual troughs measured 1.82 m in length, 0.30 m wide and 0.19 m deep with a standpipe at one end. Each was supplied with flowing raw seawater at the end opposite the standpipe. Seawater was taken directly from the tidal channel and entered the troughs at a constant rate of 1000 l daily (0.694 l min^{-1}). Flow rate, controlled by standpipes from a constant head box, was verified at least once daily.

Physicochemical Conditions

Daily measurements of pH to 0.01 unit, temperature to 0.1°C, dissolved oxygen to 0.1 mg l^{-1} and salinity to 0.1‰ were taken. Periodically, seawater from the channel was analyzed for total Mn, Zn, Pb, Cu, Ni, and Cd using a Perkin-Elmer model 403 atomic absorption spectrophotometer[1], and methods outlined in the accompanying manual.

Test Organisms

About 600 adult *Mya arenaria* were purchased from a commercial shellfish dealer in Wickford, Rhode Island, on 6 Dec., 1973. Average wet weight of drained meats, based on 25 clams, was 5.4 g. Within a few hours of purchase, 55 clams were randomly distributed in seawater along the bottom of each of the 6 troughs. The remainder were held in a 1000 l tank supplied with rapidly flowing raw seawater. Dead clams were removed daily from troughs and replaced with apparently healthy clams from the 1000 l tank. Clams were considered acclimatized when no deaths had been observed for 2 weeks; winter study clams were acclimatized on 3 January 1974.

Additional *Mya arenaria* were purchased from the same dealer on 24 May 1974; drained wet weight was within 10% of the winter shipment. There were no deaths during the next 2 weeks and clams were considered acclimatized on 7 June 1974.

Test Toxicant

One hundred liters of a distilled water solution of $MnCl_2 \cdot 4H_2O$, $ZnCl_2$, $Pb(NO_3)_2$, $CuCl_2 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$, and $CdCl_2 \cdot 2\frac{1}{2}H_2O$ were prepared to contain, in mg metal l^{-1} of stock, 9338 Mn, 3242 Zn, 90.8 Pb, 64.9 Cu, 64.9 Ni, and 1.30 Cd. All concentrations were verified by atomic absorption spectrophotometry. Using Har-

[1]Use of product or trade names mentioned throughout text does not necessarily constitute endorsement by the U.S. Environmental Protection Agency.

Table 1. Dissolved and particulate concentrations of 6 metals in seawater after passage through troughs containing *Mya arenaria*. Values are in µg l^{-1}, on 14 January, 1974, controls and 100% toxicant groups

	Dissolved						Particulate					
	Mn	Zn	Pb	Ni	Cu	Cd	Mn	Zn	Pb	Ni	Cu	Cd
Controls												
No supplement diet	7.8	10.0	<1.0	1.7	0.9	0.06	0.82	0.04	<1.0	0.33	0.01	<0.06
Supplement diet	9.8	4.9	<1.0	1.3	1.1	0.11	1.07	0.34	<1.0	0.28	0.06	<0.06
Experimental[a]												
No supplement diet	7200	2460	56.2	58.6	36.7	1.30	3.58	4.94	4.75	1.03	2.16	<0.06
Supplement diet	7300	2470	50.8	56.6	35.6	1.38	2.87	5.86	3.54	0.00	2.04	<0.06

[a]Control levels plus calculated addition of 7200 µg l^{-1} Mn^{2+}, 2500 µg l^{-1} Zn^{2+}, 70 µg l^{-1} Pb^{2+}, 50 µg l^{-1} Ni^{2+}, 50 µg l^{-1} Cu^{2+}, and 1 µg l^{-1} Cd^{2+}

vard Apparatus model 1203 peristaltic pumps (Millis, Mass 02054) and medical grade silastic tubing, two troughs received 771 ml of the stock daily (= 100% metal mixture) and two troughs received 154.2 ml daily (= 20% metal mixture); the remaining two troughs were controls. The 100% metal mixture produced calculated delivered levels above those of ambient seawater, in µg l^{-1}, of 7200 Mn, 2500 Zn, 70 Pb, 50 Cu, 50 Ni, and 1.0 Cd. The 20% mixture contained final calculated concentration levels above controls of 1440 µg l^{-1} Mn, 500 Zn, 14 Pb, 10 Cu, 10 Ni, and 0.2 Cd. Analyses of dissolved and particulate concentrations of the 6 metals in flowing seawater among controls and the 100% metal group were made by Dr. E.W. Davey of this laboratory. Results of one analysis are shown in Table 1. Dr. Davey concluded that negligible amounts of metals were lost as a result of passage through troughs containing shellfish, that metals tested remained in solution under the conditions of this experiment, and that metals tested did not sorb onto particulate materials.

Pumps were checked daily for operation; samples of water exiting from all troughs were collected weekly throughout the study for Mn and Zn determinations by atomic absorption; no analyses were made for Pb, Ni, Cu, and Cd.

Results of preliminary static acute toxicity bioassays that I conducted at 4°C demonstrated that *Mya arenaria* were extremely resistant to the toxicant mixture. No deaths were observed in 14 days during immersion in a solution containing 72 mg l^{-1} Mn, 25 mg l^{-1} Zn, 0.7 mg l^{-1} Pb, 0.5 mg l^{-1} Cu, 0.5 mg l^{-1} Cd, and 0.01 mg l^{-1} Cd. This was equivalent to 10 times the highest level actually tested in flowing water tests.

Supplemental Diet

Suppemental algal diets were offered to some groups to ascertain whether this constituted a factor in metals accumulation by *Mya arenaria*. Algae were fed to: one control, a 20% toxicant group, and a 100% toxicant group. Each group received 7 liters of algal culture (300,000 cells/ml, determined by Coulter Counter) twice weekly over a period of 3½ to 4 h. During the winter study this algal diet comprised one of the following three species: *Skeletonema costatum*, *Monochrysis lutheri*, or *Isochrysis galbana*. For the summer study, it was *I. galbana* exclusively.

Experimental Methods

Winter Study

The study on effects of two diets and two different concentrations of a metal mixture solution on *Mya arenaria* under flowing ambient seawater conditions was initiated on 4 January 1974. Troughs were inspected daily, at which time dead clams were removed, recorded, then discarded.

Immediately prior to the addition of the metal mixture, 5 apparently healthy *Mya arenaria* were removed from individual troughs (total = 30), placed in labelled polyethylene bags, and frozen at -20°C. This procedure was repeated at 24 h, and at 1, 2, 4, 6, 8, 12, and 16 weeks. After several months of storage, individual *M. arenaria* collected at three time intervals (at start, 6 weeks, and 16 weeks) were prepared for analysis of 14 elements. The remainder of the samples were archived. Clams were thawed, rinsed in deionized water, and blotted dry. The

soft parts were placed in acid-stripped (6 N HCl) beakers, weighed, dried at 95°C for 24 h, reweighed, then ashed at 425°C for an additional 24 h. On cooling, 10 ml of concentrated HNO_3 was added to each sample, redried at 95°C for 24 h, and reashed at 425°C for 24 h. Ten ml of concentrated HNO_3 was added to the cool sample, filtered through Whatman 42 paper, and diluted to 50 ml with deionized water. Acid-soluble fractions were analyzed for Cd, Ni, Cr, Pb, V, Cu, Mn, Sr, Zn, Fe, Ca, Mg, K, and Na by atomic absorption. Some elements were analyzed because they were constituents of the metal mixture (Cd, Ni, Pb, Cu, Mn, Zn), others because they may represent indicators of stress in shellfish (Na, K, Ca, Mg, Sr, Fe) as proposed by Eisler and Weinstein (1967), and still others (Cr, V) because background data on these metals in shellfish were desired by various staff members. The precision of the analyses was satisfactory; results from replicate samples were always within 2%.

For every exposure time-diet-toxicant concentration regimen, a mean and standard deviation was computed for each of the 14 elements. For each element examined, statistical analyses-of-variance tests were applied to the following hypotheses: elemental composition of controls did not vary during the 16 week study; diet did not affect elemental composition of controls; and finally, treated groups were not different from appropriate controls at the $P = <0.05$ level of significance. Data are presented in tabular form, and graphically where appropriate, using modifications of the profile technique of Eisler (1972).

Summer Study

A study similar to the winter study was initiated on 24 June 1974; however, there were several differences. For example, at the start, and also at 1, 7 and 14 days, a total of 5 live *Mya arenaria* were removed from each trough. In addition, only those groups that were subjected to the "no supplemental diet" regimen were analyzed for whole-body burdens of 14 elements. Also, each sample analyzed comprised 4 homogenized bivalves; an aliquot of about one-quarter of this was treated as in the winter study for elemental analysis. Statistic treatment was contraindicated, as there was only a single replicate per exposur time-diet-toxicant concentration-metal combination. Results were presented in tabular and graphical form.

Results

Winter Study

Physicochemical Conditions

Daily water temperature measurements during the 112 day study varied from 5.4°C at the start (3 January 1974) to 0.0°C (18 January 1974) afterwards, the temperature rose more or less progressively to 10.2°C (29 April 74). Salinity values ranged from 27.0 to 33.8‰, with most of the observations at 30 ± 1‰. The pH range was relatively narrow, with extreme values recorded during the study of 7.85 and 8.15. Dissolved oxygen was never less than 4.0 mg l^{-1}.

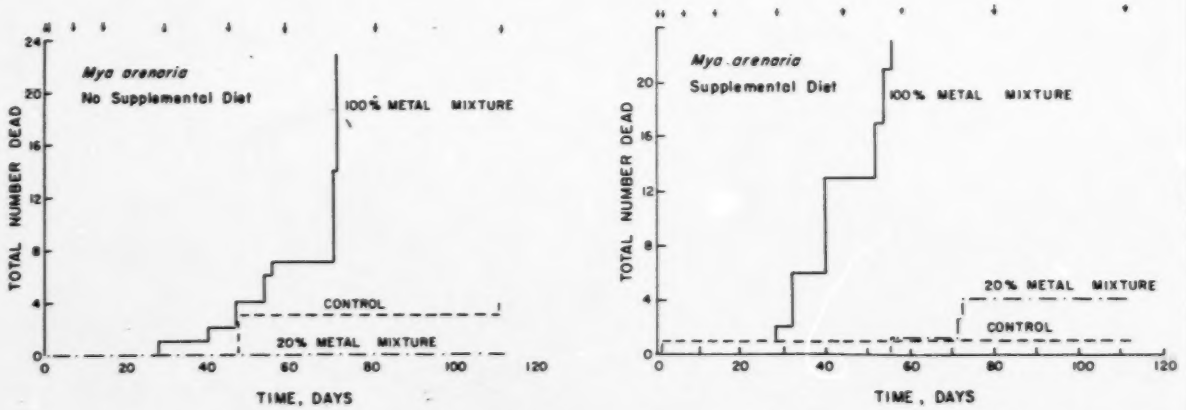

Fig. 1. *Mya arenaria*. Winter study: mortality among clams held in flowing raw seawater during exposure to 20 or 100% concentrations of a mixture of metal salts. Left: without supplemental diet; right: with supplemental diet. Arrows indicate intervals when 5 survivors were removed from each group; initial number per group was 55

Concentrations of metals in water from control troughs never exceeded the following values (µg l^{-1}): Mn, 10; Zn, 10; Ni, 1.7; Cu, 1.1; Cd, 1.1; and Pb, 1.0. Manganese and Zn concentrations in troughs receiving the 20% toxicant solution were always within 5% of calculated values. For the 100% toxicant groups, Mn and Zn levels were also within 5% of calculated values but only during the first 8 weeks of the study. Reductions in water flow to the 100% toxicant groups, caused by particulate materials clogging standpipes leading to these troughs, produced elevated levels of Mn and Zn between the 8th and 9th weeks by as much as 32% over expected concentrations; afterwards, Mn and Zn values were about 10% higher than expected.

Survival

No *Mya arenaria* exposed to the 100% metal mixture lived longer than 70 days (Fig. 1). Mortalities among clams at this test concentration fed a supplemental diet exceeded control deaths by Day 28; deaths among unfed *M. arenaria* exceeded controls by Day 30. Survival among controls and the 20% metal mixture group was high throughout the study, regardless of the diet (Fig. 1).

Whole-Body Elemental Composition

Elemental composition of control clams changed significantly during the winter study, but was relatively unaffected by diet (Fig. 2). After 6 weeks the controls, regardless of diet, contained significantly more Ni, Cu, Na, K, Cd, and Mn, and significantly less Fe than at the start (Fig. 2). After 16 weeks, there were significant and consistent increases in Ni, Cu, and Cd, and consistent decreases in K, Fe, Cr, Mn, Ca, V, and Sr compared to identical parameters 16 weeks earlier (Fig. 2). These observations demonstrated clearly that experimentals must be compared to controls of identical time-diet regimen.

After 6 weeks of exposure to the 20% toxicant solution, survivors in both fed and unfed groups, compared to controls, consistently exhibited 150 to 500% more Zn, Pb, Fe, and Mn; 25 to 300% more Cu, Mg, and Cd; and about 25% less Ni (Fig. 3). By the 16th week, survivors immersed in the 20% solution, regardless of diet, exhibited a sharply-defined pattern: 2 to 5 times more Cu, Zn, Pb, and Mn than controls and comparatively negligible changes in other elements (Fig. 3). Profiles of *Mya arenaria* surviving exposure to the 100% metal mixture for 6 weeks (Fig. 4) were, with two exceptions, remarkably similar to those exposed to the lower concentration of 20% for the longer period of 16 weeks. The exceptions were the elevated levels of Ni in the 100% group (3x more than controls) and also the higher absolute concentrations of Cu, Zn, Mn, Pb, and Ni (10 to 19x greater) compared to the appropriate controls (Fig. 4).

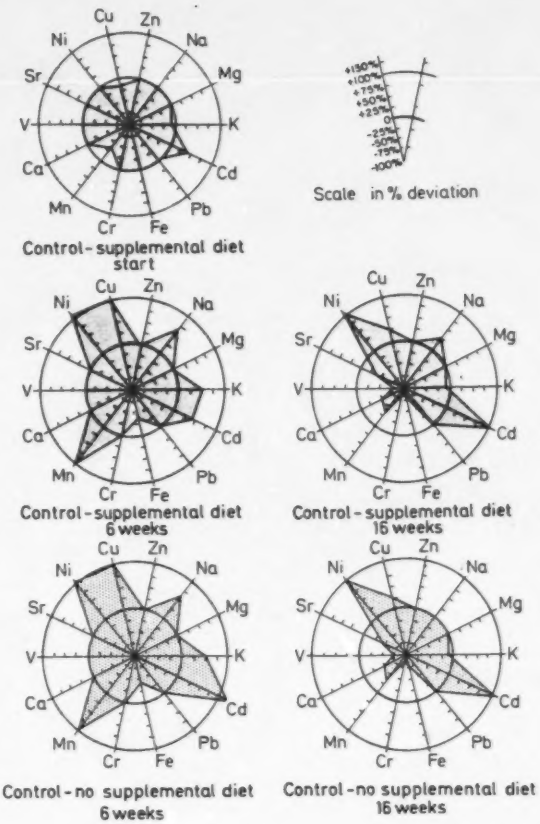

Fig. 2. *Mya arenaria*. Profiles of control clams under two dietary regimes at 3 time intervals: start, 6 weeks, and 16 weeks. Values are in significant percent deviation from controls without supplemental diet at start: any deviation from zero is statistically significant at the 0.05 probability level or lower. Mean (standard deviation) values of individual metals for controls without supplemental diet at start, in mg/kg dry weight, were: Cd, 0.34 (0.14); Ni, 2.15 (1.46); Cr, 4.4 (3.9); Pb, 6.6 (9.1); V, 8.4 (2.9); Cu, 9.8 (1.5); Mn, 29 (5); Sr, 63 (16); Zn, 93 (35); Fe, 4,834 (2,593); Ca, 10,522 (4,157); Mg, 11,609 (3,194); K, 13,936 (1,307); Na, 73,970 (15,235)

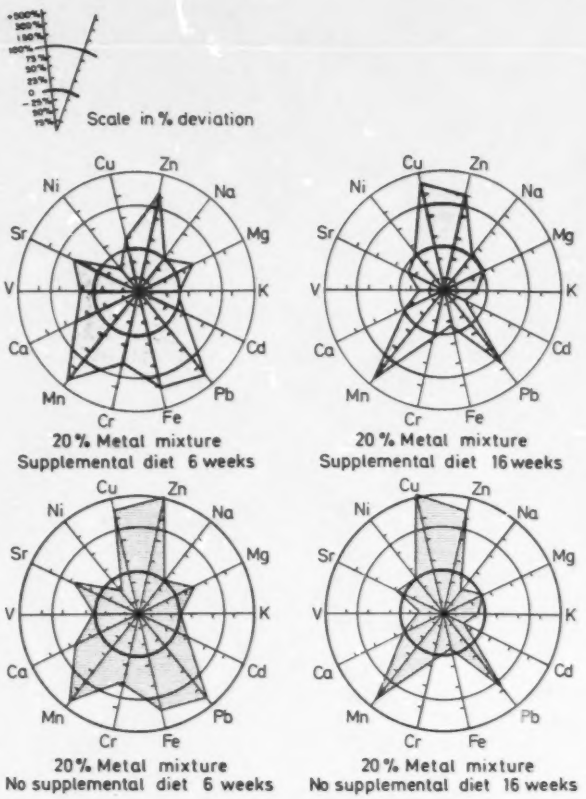

Fig. 3. *Mya arenaria*. Profiles of clams under two dietary regimes exposed to 20% metal mixture for 6 or 16 weeks. Values are in significant percent deviation from controls of identical diet-time regimen; any deviation from zero is statistically significant at the 0.05 probability level or lower

Bioaccumulation

Mean content of some metals in *Mya arenaria* exposed experimentally to low environmental levels (this study) were higher, sometimes substantially higher, than mean values for *M. arenaria* taken from productive commercial shellfish beds (Pringle *et al.*, 1968). In almost every instance, average values for Ni, Pb, Cu, Mn, and Zn exposed to a 20% toxicant mixture or higher for 6 weeks or longer were greater than those from field populations (Table 2). Values for Pb, Mn, and Zn in certain cases exceeded the upper limits of the range reported for this species by Pringle *et al.* (1968) (Table 2). However, the upper limit recorded for copper of 90 mg kg^{-1} wet weight by Pringle *et al.* (1968) is not consistent with copper residue determinations in *M. arenaria* under the conditions of the present study.

Average concentration factors of selected metals by *Mya arenaria* from the medium under laboratory (this study) and field (Pringle *et al.*, 1968) conditions are shown in Table 3. Clams subjected to the 20% toxicant mixture exhibited lower concentration factors at 16 weeks than at 6 weeks for Zn, Mn, and Pb, suggesting a detoxification or equilibrium mechanism; but Cu and Ni continued to accumulate at approximately the same high rate during exposure for 6 weeks in the 100% toxicant solution (Table 3).

Summer Study

Physicochemical Conditions

During the acclimatization period from 24 May 1974 and the time toxicant was

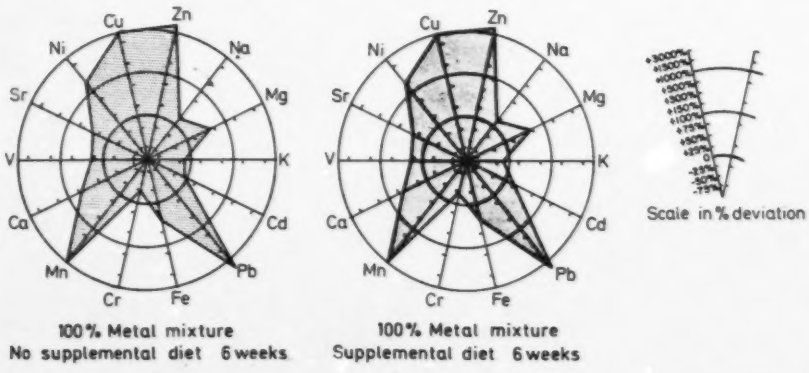

Fig. 4. *Mya arenaria*. Profiles of clams unter two dietary regimes after exposure to 100% metal mixture for 6 weeks. Values are in significant percent deviation from appropriate controls; any deviation from zero is statistically significant at the 0.05 probability level or lower. There were no survivors from these groups after the 10th week

Table 2. *Mya arenaria*. Comparison of mean concentration of Ni, Pb, Cu, Mn, and Zn in samples from this study (winter portion) with those from productive shellfish beds (after Pringle *et al.*, 1968). Values are in mg metal per kg wet weight soft parts

Metal	Pringle *et al.* Mean (range)	This study[a] Controls	20% mixture 6 weeks	20% mixture 16 weeks	100% mixture 6 weeks
Nickel	0.27 (0.1-2.3)	0.39	0.32	0.49	1.26
Lead	0.70 (0.1-10.2)	0.68	2.94	1.60	13.34
Copper	5.80 (1.2-90.0)	1.44	5.42	7.04	24.66
Manganese	6.70 (0.1-29.9)	3.58	31.31	10.40	66.01
Zinc	17.00 (9.0-28.0)	9.57	48.35	31.20	140.47

[a]Based on average wet:dry ratio of 9.71 for *M. arenaria* flesh (range 8.66 to 11.22).

Table 3. *Mya arenaria*. Average concentration factors of Zn, Cu, Mn, Pb, and Ni by clams from productive shellfish beds (after Pringle *et al.*, 1968) and from winter portion of this study

Metal	Pringle *et al.*	This study[a] Controls	20% mixture 6 weeks	20% mixture 16 weeks	100% mixture 6 weeks
Zinc	1700	>957	77.6	43.3	52.4
Copper	2000	>1600	398.0	560.0	464.0
Manganese	3350	>358	19.3	4.7	8.7
Lead	3400	>680	161.4	65.7	180.8
Nickel	4250	>229	0.0	10.0	17.4

[a]Concentration factor = mg metal/kg wet weight (experimental minus control value)/mg metal per liter medium (experimental minus control).

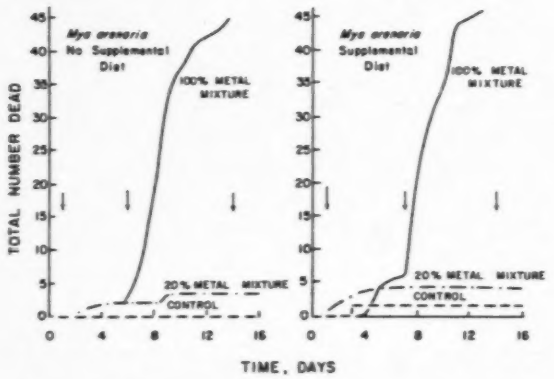

Fig. 5. *Mya arenaria*. Summer study: mortality among clams held in flowing raw seawater during exposure to 20 or 100% concentrations of a mixture of metal salts. Left: without supplemental diet; right: with supplemental diet. Arrows indicate intervals when 5 survivors were removed from each group; initial number per group was 55

added on 24 June 1974, the water temperature rose progressively from 12.0° to 16.5°C. During the dosing portion, the mean daily temperature rose more or less consistently from 16.5° to 22.5°C. Salinity during the 16 day study ranged between 29.0 and 32.3‰, pH between 7.90 and 8.20, and dissolved oxygen between 7.0 and 8.2 mg l^{-1}. Concentrations of total Mn and Zn in all troughs were determined daily by atomic absorption spectrophotometry, and were always within 5% of expected values. Concentrations of Pb, Ni, Cu, and Cd in control seawater were determined weekly and, as was true for Mn and Zn, did not exceed values reported in the winter study.

Survival

Deaths were recorded among *Mya arenaria* held in the 100% toxicant solution starting at about 96 h; all *M. arenaria* receiving this treatment, regardless of diet, were dead by Day 14 (Fig. 5). Those immersed in the 20% toxicant solution exhibited slightly higher mortality than controls during the first 6 to 9 days, but no deaths were observed afterwards in either the 20% group or in controls (Fig. 5).

Whole-Body Elemental Composition

Because analysis of data from the winter portion suggested only minor differences in elemental composition as a result of supplemental feeding, only those groups held in raw seawater without receiving a supplemental diet were analyzed. Table 4 lists the concentrations of 14 elements in control *Mya arenaria* and those subjected to various toxicant concentrations for 1, 7 and 14 days.

As was true in the winter experiment, values for some elements among controls varied substantially with time during the study (Table 4). As a result, profiles of test groups were compared to controls of identical time frames. In the 20% group, Mn levels were more than 2 times those of controls by 24 h, with minor changes in other parameters (Fig. 6). However, profiles of survivors from the 100% toxicant group exhibited striking changes at only 24 h; *Mya arenaria* in this group contained about 7 x more Mn, 5 x more Pb, 4 x more Zn, 3 x more Ni, and 2 x more Cu than controls (Fig. 6).

By 7 days, the 20% group contained about 13 x more Mn, 6 x more Zn, 4 x more Pb, and 2 x more Cu and Ni than controls (Fig. 7). After 14 days, survivors exposed to the 20% metal mixture still contained the same approximate levels of Mn (12 x), Zn (7 x), and Ni (3 x) compared to controls; however, Pb content was now about 7 x higher than controls and Cu about 4 x greater (Fig. 7). After 7 days, profiles of the 100% toxicant group showed Mn and Ni content essentially unchanged from those at 24 h (now 7 x and 3 x, respectively, greater than controls), but those for Pb, Cu, and Zn showed dramatic increases to 25 x, 13 x, and 11 x, respectively (Fig. 7).

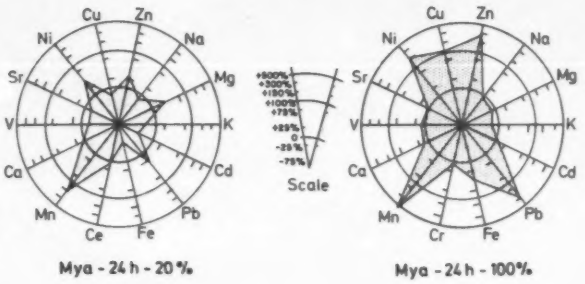

Fig. 6. *Mya arenaria*. Summer study: profiles of clams surviving immersion for 24 h in 20 or 100% metal mixture. Values are in percent deviation from controls of identical diet-time regimen

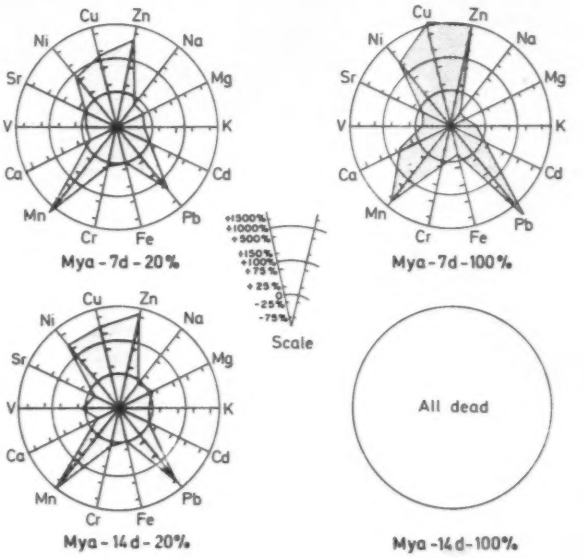

Fig. 7. *Mya arenaria*. Summer study: profiles of clams surviving exposure for 7 or 14 days in 20 or 100% metal mixture. Values are in percent deviation from controls of identical diet-time regimen

Bioaccumulation

Mean concentrations of Ni, Pb, Cu, Mn, and Zn in *Mya arenaria* exposed to a 100% metal mixture for 7 days were substantially higher than values reported for *M. arenaria* collected from high-density commercial shellfish beds; exposure for only 24 h to the 100% mixture produced elevated levels among 4 of the 5 metals (Table 5). Immersion in a 20% toxicant mixture for 24 h or longer produced substantial and consistent increases in whole body Pb, Cu, Mn, and Zn over control values (Table 5). As was true in the winter study, certain treatments produced metal accumulations in *M. arenaria* that exceeded the upper ranges reported by Pringle *et al.* (1968) for this species on productive commercial beds.

For example, groups subjected to the 100% toxicant mixture for 7 days exceeded the range for lead reported by Pringle *et al.* (1968); similar cases occurred for Mn and Zn in *M. arenaria* held 24 h or longer in a 100% toxicant solution, and for Mn and Zn in *M. arenaria* held 7 days or longer in a 20% toxicant solution (Table 5).

Concentration factors for *Mya arenaria* in the summer study are listed in Table 6. Zinc, Cu, Pb, and Ni continued to accumulate in *M. arenaria* with increasing exposure time, and this is apparent in

Table 4. *Mya arenaria*. Effect of toxicant concentration on mean content of selected metals in clams surviving exposure in flowing seawater for various intervals. Values shown are in mg element per kg dry weight soft parts. Exposures initiated 24 June 1974 at ambient water temperatures

Days of exposure and toxicant concentration[a]	Cd	Ni	Cr	Pb	V	Cu	Mn	Sr	Zn	Fe	Ca	Mg	K	Na
0														
None	0.6	4.1	2.0	7.6	5.8	14.6	43	124	127	230	12873	16001	10436	105587
1														
None	0.6	1.8	1.9	7.0	7.4	14.3	70	126	98	317	13793	15886	10559	107970
20%	0.4	2.5	2.1	9.0	6.2	12.2	167	109	127	173	14078	16051	8727	102458
100%	0.6	4.7	2.1	32.2	9.1	29.7	503	133	422	509	14953	14953	9110	97269
7														
None	0.5	2.6	1.9	6.7	8.0	11.8	63	149	102	308	16867	19269	9662	122876
20%	0.6	4.8	2.2	24.2	7.0	26.0	799	148	577	323	14670	16142	8324	104855
100%	0.6	8.2	2.2	173.5	5.7	135.0	444	135	1104	265	25224	10294	7855	63913
14														
None	0.6	2.3	1.8	7.5	6.7	12.0	61	163	109	282	20125	16535	10062	106694
20%	0.6	6.7	2.2	55.7	7.2	49.4	738	124	804	280	12741	15379	9612	99248

[a]See text for composition of toxicant solution.

Table 5. *Mya arenaria*. Comparison of mean concentrations of Ni, Pb, Cu, Mn, and Zn in clams from this study (summer portion) with those from productive shellfish beds (after Pringle *et al.*, 1968). Values are in mg metal per kg net weight soft parts

Metal	Pringle *et al.*	This study[a]					
		Controls	20% mixture			100% mixture	
			1 day	7 days	14 days	1 day	7 days
Nickel	0.27	0.28	0.26	0.49	0.24	0.48	0.84
Lead	0.70	0.74	0.93	2.49	5.74	3.32	17.86
Copper	5.80	1.36	1.26	2.68	5.09	3.06	13.90
Manganese	6.70	6.10	17.20	82.28	76.00	51.80	45.72
Zinc	17.00	11.22	13.08	59.42	82.80	43.36	113.70

[a]Based on average wet:dry ratio of 9.71.

Table 6. *Mya arenaria*. Average concentration factors of Zn, Cu, Mn, Pb, and Ni by clams from productive shellfish beds (after Pringle *et al.*, 1968) and from summer portion of this study

Metal	Pringle *et al.*	This study[a]					
		Controls	20% mixture			100% mixture	
			1 day	7 days	14 days	1 day	7 days
Zinc	1700	≥1122	3.7	96.4	143.1	12.9	41.1
Copper	2000	≥1511	0.0	132.0	373.0	34.0	250.8
Manganese	3350	≥610	7.7	52.9	48.5	6.3	5.5
Lead	3400	≥740	13.6	125.0	357.1	36.8	244.6
Nickel	4250	≥165	0.0	21.0	0.0	4.0	11.2

[a]Concentration factor = mg metal per kg wet weight (experimental minus control value)/mg metal per liter medium (experimental minus control).

Table 7. Lethality of a synthetic metal mixture to 0, 50, and 100% of adults from 5 marine species. Assays conducted at 30‰ S, 4°C, pH 8.0, under static conditions. Values are in multiples of total mixture at designated time intervals. 1.0 metal mixture contained 7200 µg l^{-1} of Mn^{2+}, 2500 of Zn^{2+}, 70 of Pb^{2+}, 50 of Cu^{2+}, 50 of Ni^{2+}, and 1 µg l^{-1} of Cd^{2+}

Species	Time (days)	LC_0	LC_{50}	LC_{100}
Rock crab, *Cancer irroratus*	4	1.0	3.0	10.0
	10	3.0	5.5	10.0
Mummichog, *Fundulus heteroclitus*				
Softshell clam, *Mya arenaria*	14	10.0	>10.0	>10.0
Quahaug clam, *Mercenaria mercenaria*	14	10.0	>10.0	>10.0
Mahogany clam, *Arctica islandica*	14	10.0	>10.0	>10.0

both 20 and 100% toxicant mixtures. Only Mn exhibited a marked reduction in concentration factor with time, and this was true for clams exposed to both high and low toxicant mixtures. Clams subjected to the 20% mixture for only 14 days in summer exhibited substantially higher concentration factors for Zn, Mn, Pb, and Ni than clams exposed to the same mixture for 6 weeks during winter. In general, it would appear that accumulation of all metals proceeds at a faster rate at elevated temperatures and that this is especially pronounced for lead.

Discussion

Bivalve molluscs are more resistant to the mixture of metal salts tested than other groups of marine macrofauna for which data are available. Results of unpublished static acute toxicity tests that I conducted at 4°C ± 2 C°, 30‰ S and pH 8.0 demonstrated that the softshell clam, the quahaug clam *Mercenaria mercenaria*, and the mahogany clam *Arctica islandica* after 14 days were all at least 3 times more resistant than the mummichog *Fundulus heteroclitus*, an estuarine teleost, and at least 10 times more resistant than the adult rock crab *Cancer irroratus* (Table 7). Bioassays with various species of algae and zooplankters at different thermosaline regimes suggest that these groups are even more sensitive than adult rock crab to the same metal mixture (Dr. J. Gentile, EPA Narragansett, R.I., personal communication).

The toxicant mixture examined herein contained the highest concentrations of individual metals actually measured from surficial interstitial sediment waters at 17 stations, in mid-Narragansett Bay, Rhode Island (Eisler *et al.*, unpublished data). However, it is emphasized that other metals were also present in sediment waters, most notably iron at concentrations up to 559 µg l^{-1} (Eisler *et al.*, unpublished data). For this and other reasons, it was not possible to conclude at this time that metals in surficial sediment waters will become available to benthic fauna as a result of perturbations from storms, local dredging practices, or other sources. It is more probable that some loss in biological availability will occur through the scavenging action of iron, and from naturally-occurring chelators. At present, however, the extent and degree of this phenomenon is unknown.

In this study, all *Mya arenaria* subjected to the 100% toxicant mixture during winter were dead by the 10th week of exposure. They were more sensitive than other species of bivalves which were tested under identical conditions: *Arctica islandica* exhibited about 40% mortality in 16 weeks while all *Mercenaria mercenaria* survived the 16 week exposure (Eisler, in preparation). A similar pattern was recorded for the summer study: all *Mya arenaria* exposed to the 100% mixture were dead in 16 days; no deaths were recorded in 16 days among *Mercenaria mercenaria* receiving identical treatment. Results of static acute toxicity bioassays conducted by Eisler (1977) with *Mya arenaria* at 22°C and 30‰ S demonstrated that copper and, to a much lesser extent, zinc could account for all deaths at concentrations tested within the mixture. LC_{50} (168 h) values, in mg l^{-1} metal added at the start, were 0.035 for Cu, 0.150 for Cd, 1.55 for Zn, 8.8 for Pb, 300.0 for Mn, and >50.0 for Ni. Additional tests were conducted with Zn^{2+} and Cu^{2+} during fall (17°C) and winter (4°C): *M. arenaria* showed increasing resistance with decreasing temperature (Eisler, 1977). For Cu the LC_{50}'s at 17° and 4°C were 0.086 and >3.0 mg l^{-1}, respectively; for Zn these were 2.65 and >25.0 mg l^{-1}, respectively (Eisler, 1977). Similar results were obtained with *A. islandica*: Cu^{2+} was several orders of magnitude more toxic to this species in 168 h at 10°C than Zn^{2+}, Cd^{2+}, Ni^{2+}, or Pb^{2+} (Eisler, in preparation). Results of these studies are in good agreement with the present investigation and demonstrate the importance of seasonal temperature differences in evaluating toxicity. These findings suggest that local dredging practices should be curtailed during mid-summer.

when benthos are apparently most sensitive to perturbations, and resumed in late-fall or early winter when effects appear diminished.

Changes in elemental composition of control *Mya arenaria* observed during the present 16 week study were not unexpected. Marine bivalve molluscs are known to exhibit consistent and measurable changes in content of selected metals with increasing growth (Boyden, 1974; MacKay *et al.*, 1975; Eisler *et al.*, in review); with changing seasons (Frazier, 1975); and as a reflection of metal content of sediment substrate (Frazier, 1976). In this study, *M. arenaria* showed a pronounced decrease between January and late April in whole-body content, especially for Fe, but also for Mn, Cu, K, Cr, Ca, Sr, and V. Frazier (1975), in his studies with the American oyster *Crassostrea virginica*, observed a marked decrease in whole-body Fe and Mn between October and December, with increases in Zn, Cu, and Cd during this same period. No significant change in Zn content was observed in *M. arenaria* during the present study, whereas Cd increased during the same interval. It appears that if metal concentrations are to become useful indicators of toxicant-induced stress in bivalves, as proposed by Eisler and Weinstein (1967), it is necessary for elemental levels to be documented on a variety of growth stages throughout the year and that the role of metals in normal growth and metabolism be ascertained. This subject area appears to warrant additional and intensive research effort.

Feeding a supplemental diet of algae had little measurable effect on survival or elemental composition of *Mya arenaria* under the conditions of this study. It is possible that the total amount of algae offered experimentally was negligible in terms of concentrations available in raw seawater; unfortunately, no chlorophyll *c* or other measurements were made.

Within the constraints of this study, I concluded that adults of *Mya arenaria* can biomagnify potential environmental levels of Mn, Zn, Cu, Pb and, to a lesser extent, Ni; that accumulations and death rates were accelerated at higher temperatures; and that in some cases final concentrations of individual metals greatly exceeded those reported in *M. arenaria* from productive commercial shellfish beds. In all cases, the probable cause of death was due to copper toxicity although zinc at the levels tested may have had some influence. The significance of the changes observed in content of Fe, Ca, Sr, and other elements in *M. arenaria* controls during the study period is not fully understood, but may have affected survival and accumulation patterns; this research area clearly merits additional effort modeled along the lines established by Frazier (1975, 1976).

Acknowledgements. I am obligated to E. Davey and A. Soper for water chemistry analyses and preparation of toxicant solutions; to M. Wapner, G. Hartson, and T. White for maintaining troughs and preparing samples for elemental analysis; to W. Galloway and P. Rogerson for atomic absorption analyses; to F. Jacoff for algal cultures; to (the late) R. Johnson for facilities construction; and to N. Goldberg for computer services.

Literature Cited

Boyden, C.R.: Trace element content and body size in molluscs. Nature, Lond. *251*, 311-314 (1974)

Cusick, J.: Mucous cell response of the guppy to heavy metals and water quality, 143 pp. Cincinnati, Ohio: Ph. D. Thesis, University of Cincinnati 1967

Eaton, J.G.: Chronic toxicity of a copper, cadmium, and zinc mixture to the fathead minnow (*Pimephales promelas* Rafinesque). Wat. Res. *7*, 1723-1736 (1973)

Eisler, R.: Pesticide-induced stress profiles. *In*: Marine pollution and sea life, pp 229-233. Ed. by M. Ruivo. London: Fishing News (Books) Ltd. 1972

- Annotated bibliography on biological effects of metals in aquatic environments, 287 pp. Washington, D.C.: U.S. Government Printing Office 1973 (U.S. Environmental Protection Agency Rep. No. R3-73-007)
- Acute toxicities of selected heavy metals to the softshell clam, *Mya arenaria*. Bull. envir. Contam. Toxicol. *17*, 137-145 (1977)
- , M.M. Barry, R.L. Lapan Jr., G. Telek, E.W. Davey and A.E. Soper: Metals survey of the marine clams *Pitar morrhuana*, collected near a Rhode Island (USA) electroplating plant. (In review)
- and G.R. Gardner: Acute toxicology to an estuarine teleost of mixtures of cadmium, copper, and zinc salts. J. Fish Biol. 5, 131-142 (1973)
- , R.L. Lapan, Jr., G. Telek, E.W. Davey, A. E. Soper and M.M. Barry: Survey of metals in sediments near Quonset Point, Rhode Island. Mar. Pollut. Bull. (In press)
- and M. Wapner: Second annotated bibliography on biological effects of metals in aquatic environments, 400 pp. Springfield, Virginia: National Technical Information Service 1975 (U.S. Environmental Protection Agency Rep. No. 600/3-75-008)

- and M.P. Weinstein: Changes in metal composition of the quahaug clam, *Mercenaria mercenaria*, after exposure to insecticides. Chesapeake Sci. *8*, 251-258 (1967)

Frazier, J.M.: The dynamics of metals in the American oyster, *Crassostrea virginica*. I. Seasonal effects. Chesapeake Sci. *16*, 162-171 (1975)

- The dynamics of metals in the American oyster, *Crassostrea virginica*. II. Environmental effects. Chesapeake Sci. *17*, 188-197 (1976)

Gray, J.S. and R.J. Ventilla: Growth rates of sediment-living marine protozoan as a toxicity indicator for heavy metals. Ambio *2*, 118-121 (1973)

Lloyd, R.: The toxicity of mixtures of zinc and copper sulfates to rainbow trout (*Salmo gairdneri* Richardson). Ann. appl. Biol. *49*, 535-538 (1961)

MacKay, N.J., R.J. Williams, J.L. Kaprzac, M.N. Kazacos, A.J. Collins and E.H. Auty: Heavy metals in cultivated oysters (*Crassostrea commercialis = Saccostrea cucullata*) from the estuaries of New South Wales. Aust. J. mar. Freshwat. Res. *26*, 31-46 (1975)

Pringle, B.H., D.E. Hissong, E.L. Katz and S.T. Mulawka: Trace metal accumulation by estuarine molluscs. J. sanit. Engng Div. Am. Soc. civ. Engrs *94 SA3*, 455-475 (1968)

Sprague, J.B.: Avoidance of copper-zinc solutions by young salmon in the laboratory. J. Wat. Pollut. Control Fed. *36*, 990-1004 (1964a)

- Lethal concentrations of copper and zinc for young Atlantic salmon. J. Fish. Res. Bd Can. *21*, 17-26 (1964b)

- and B.A. Ramsey: Lethal levels of mixed copper-zinc solutions for juvenile salmon. J. Fish. Res. Bd Can. *22*, 425-538 (1965)

Dr. R. Eisler
U.S. Environmental Protection Agency
Environmental Research Laboratory
Narragansett, Rhode Island 02882
USA

Date of final manuscript acceptance: May 20, 1977. Communicated by M.R. Tripp, Newark